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MANUAL
OF
PHYSIOLOGY

WILLIAM D. ZOETHOUT, Ph. D.

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Laboratory Manual OF PHYSIOLOGY

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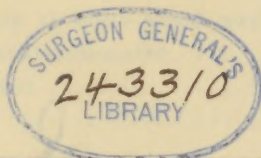
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Chicago, Ill.



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OF
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PART ONE

Apparatus

Exp. 1—Galvanic, Voltaic or Constant Current.

Apparatus:—Plug-key, wire, simple key, litmus paper.

Insert the plug-key in the socket marked "Stimulating Current." Connect one of the wires of the plug-key with a simple electric contact key and attach wires as indicated in Fig. 1. The ends of the wires should be bright and all contacts made firm.

- (a) When you have set up the apparatus as directed, proceed as follows: Moisten a strip of blue litmus paper with physiological salt solution (0.7% NaCl). Lay this on a clean glass plate and hold the two electrodes on the paper about $\frac{1}{2}$ cm. apart. Close the key and let the current flow for say one minute. Notice the change in color at one of the electrodes. Which one?

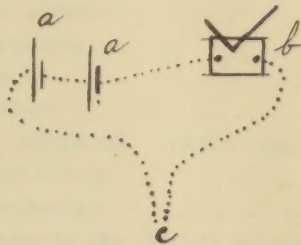


Fig. 1. Arrangement for galvanic current. *a* and *a*, plug-key; *b*, simple key; *c*, electrodes.

- (b) Repeat the above with red litmus paper. Result? Explain fully (a) and (b). Of what value is this?
- (c) Place the electrodes on the tongue; close the key and keep it closed for 5 or 6 seconds. Effect? Was the shock stronger at make or break or during the passage of the current?

Exp. 2—The Faradic or Induced Current.

Apparatus:—Plug-key, wire, key, induction coil, platinum electrodes.

The important parts of the induction coil are the primary coil (inner coil) and the secondary coil (outer coil). Note that there is no metallic connection between them. Hence any galvanic current passing through the primary can not enter the secondary coil.

Also notice the binding posts on the head piece to which the primary coil is attached. The horizontal metal bars with which the ends of the secondary coil are connected end in two binding posts. Notice the short-circuiting key near these binding posts.



Fig. 2. The induction coil. *a*, plug-key; *b*, key; *c*, electrodes; *d*, primary coil; *e*, secondary coil.

(*a*) Arrange apparatus as indicated in Fig. 2, connecting the wires of the stimulating circuit (primary circuit) with the binding posts on the inductorium marked single induction shocks (the left hand and central binding posts of the machine of The Harvard Apparatus Company). Open the short-circuiting key. Draw secondary coil away from the primary coil (about 12 cm.). Place the electrodes on the tongue; close the key in the primary circuit, keep it closed for about 3 seconds; open the key; if there is no effect, push the secondary coil one cm. closer to the primary and repeat. Continue this process until at a certain place a shock is felt when the key in the primary circuit is opened (this is called the "break" induction current). Is there any faradic current during the flow of the galvanic current through the primary coil? Bring the secondary coil gradually closer to the primary coil and repeat. Before any other results take place, the "break" shock may become too painful. You will proceed as follows:

1. Key open, electrodes on the tongue.
 2. Close the key.
 3. Take electrodes from the tongue and after that break the current. This will prevent you from taking the break shock. In this manner determine the first point where the slightest "make" shock can be felt. Record these points.
- (*b*) If the induction coil you have is so constructed that the secondary coil can be rotated either on a horizontal or vertical axis, determine what effect such rotation has on the strength of the induced current.

In what ways can the strength of the induced current be varied?

- (c) **Repeated Induction Shocks.** The shocks obtained in (a) are sometimes spoken of as single induction shocks. By an arrangement found on the front of the induction machine, the galvanic current in the primary circuit can be made and broken automatically and at every such make and break an induction shock is obtained. Connect the plug-key with the induction coil as shown by the instructor. On closing the key in the primary circuit, the galvanic current magnetizes the iron rod and thereby draws the spring away from the point of contact and the current is broken. Place the electrodes between thumb and finger (sec. coil at about 6 or 8 cm.) and notice the succession of induction shocks. These are called "repeated induction shocks." Trace the current from the plug-key through the primary coil of the inductorium, back to the plug-key. Make a diagrammatic sketch of the inductorium showing the various binding posts, the two coils and the automatic interrupter. Indicate also which binding posts are used for single and which for repeated induction shocks, and by means of arrows indicate the pathway of the current.

Exp. 3—Short Circuiting the Induced Current.

Apparatus:—Same as in Exp. 2.

Set up apparatus for single induction shocks [Exp. 2 (a)]. Place secondary coil at about 9 cm. Close key in primary circuit and a break and perhaps also a make induction shock will be felt if the electrodes are placed on the tongue. Now close the short circuiting key found on the inductorium and having the electrodes on the tongue close and open the key in the primary circuit. Did you obtain any induction shocks? Why? Trace the induced current.

- (a) Frequently it is necessary to employ only make induction shocks. This can be accomplished in the following manner:—

1. Place the electrodes on the tongue, have the short circuiting key open, close the key in the primary circuit (the secondary coil must be close to the primary coil). Notice effect. Keep electrodes on the tongue.
2. The electrodes being on the tongue, close the short circuiting key and break the primary circuit. Any break shock felt? Why? Repeat this until you are familiar with it. If you did not obtain a make induction shock, increase the strength of the induced current.

- (b) If only break shocks are wanted, how would you proceed? Prove it by experiment. N. B.—Remember the break shocks are stronger than the make shocks.

Exp. 4—The Kymograph.

Apparatus:—Kymograph.

Study the following:

- (a) How to wind the clockwork,
- (b) How to regulate the speed of the drum (two methods),
- (c) How to mount and smoke the paper on the drum,
- (d) How to label your tracings, (See below)
- (e) How to varnish (fix) and dry the tracing.

Note on labeling the tracing. If the experiment calls for "Individual Tracing," each student in the group must procure the tracing for his note book. If a "Group Tracing" is wanted, one tracing for the whole group (but bearing the names of all members participating in the experiment) is sufficient; one of the members of the group places this tracing in his note book and the others make mention of this in their books.

Each tracing must be labeled as follows:—

1. Proper lettering (*a*, *b*, *c*, etc.) in the individual curves, showing the characteristic parts of the curve, so you can call attention to these in your description,
2. Proper legend, stating the nature of the curve,
3. Your name,
4. Date,
5. No tracing is to be placed in the note book before it has been approved by the instructor. The tracing is to be permanently attached (not by clips) at the proper place in your note book.

Exp. 5—The Electro-Magnetic Signal or Time Marker.

Apparatus:—Electro-magnetic signal, key, kymograph, stand and clamp holder, plug-key, wire.

- (a) The signal magnet consists of two coils of wire above which is placed a flat piece of iron. When the galvanic current passes through these coils, the iron cores inside of the coils become magnetized and the iron plate above them is drawn down, remaining in this position until the current is broken. This causes the arm attached to the plate to move up and down and if a writing lever is attached to this arm the movements can be recorded on the kymograph.
- (b) Place the signal magnet on a stand: attach a lever; adjust, by cement, a writing pen; connect with the "clock current," inserting a simple key. Smoke the paper on the kymograph; adjust the pen of the signal magnet on the paper. Let the drum revolve at a medium speed, close the key and record seconds. Notice the tracing; label it: "Time in seconds." How many millimeters did the drum move in 10 seconds? Increase the speed considerably and repeat. Individual tracing.

Exp. 6—The Tuning Fork.

Apparatus:—Kymograph, tuning fork and starter, stand and clamp holder.

This tuning fork makes 100 double vibrations per second. Mount it on a stand and by means of the "starter" set it vibrating. Attach a writing point to one of the prongs of the fork; let the pen of the fork write on the drum and start the fork (fastest speed of drum). In order to obtain sufficient speed, it may be necessary to twirl the drum by hand (ask for instructions). When you have obtained a good tracing label it "Time in $1/100$ sec." Varnish it. How far did the drum move in $\frac{1}{4}$ second? How many hundredths of a second to the centimeter? Individual tracing.

PART TWO

Irritability, Conductivity, Contractility

Exp. 7—Forms of Stimuli.

Apparatus:—Pithing needle, glass plate, frog, heavy wire, .8% NaOH in physiological salt solution.

Pith the brain and spinal cord of a frog and dissect the gastrocnemius muscle with the sciatic nerve and the femur according to directions given by the instructor. Be very careful not to injure either the muscle or its nerve and keep them moist with 0.7% NaCl solution. Unless otherwise stated a muscle-nerve preparation consists of the gastrocnemius muscle, the sciatic nerve, and the femur. A muscle preparation consists of the gastrocnemius muscle and the femur.

Using the muscle as an indicator, determine whether the following stimuli affect the nerve:—

- (a) Thermal—apply a thick hot wire to the cut end of the nerve.
- (b) Mechanical—cut off the end of the nerve.
- (c) Chemical—dip the end of nerve in dilute NaOH solution.
- (d) Electrical—take up in the next experiment.

Exp. 8—Stimulation by the Voltaic Current.

Apparatus:—Kymograph, simple key, muscle holder and lever, wire, weights, stand, holders, frog, plug-key.

Make a muscle preparation. Place it in the muscle holder, attach the lever (use a small weight to slightly stretch the muscle). Connect one of the wires of the plug key with a simple key and from there make connection with binding post of the muscle holder. Wind a very thin copper wire around the tendon and connect this with the other wire of the plug-key, but do not let the weight of the heavy wire drag on the lever (wind the heavy wire around the stand). Ascertain that the pen writes correctly on the slow drum.

Close the key and keep it closed for about three seconds; open the key. Most likely two contractions (twitches) are obtained. Do they differ in size? What three physiological properties of the muscle were here brought into play? Define each of them. Individual tracing.

Exp. 9—Strength of Stimulus and Height of Contraction.**Single Make and Break Induction Shocks.**

Apparatus:—Kymograph, stand, muscle clamp and lever, key, induction coil, frog, plug-key, wire.

Set up apparatus for single induction shocks (Exp. 2). Use the muscle preparation of Exp. 8; connect this in the secondary circuit. Place secondary coil as far from primary as possible and stimulate the muscle by closing the key; let the key remain closed for 2 or 3 seconds (stationary drum). If a contraction occurs, move the drum forward a little, so that the contraction writes a vertical line, and no two contractions are superposed. Break the current. After each break move secondary coil one half cm. nearer to the primary coil and wait one minute before the next contraction is made. When the induced current is strong enough, a make or break contraction appears. Continue to increase the strength of the induced current till the secondary and primary coils are flush. Below the curves write M and B, and also the distance between the primary and secondary coil. Group Tracing. Note the minimal break contraction. What point of the irritability does this give? Note the first maximal contraction. What can you say of the strength of the make and break induction shock? Discuss the height of contraction. Plot a curve showing relation between the strength of the stimulus and the height of the contraction, using the distance between secondary and primary coils as abscissae and the height of the tracing in millimeters as ordinates.

Which of the four forms of stimuli listed in Exp. 7 do you regard as the most efficient and most practical? Give four reasons for this.

Exp. 10—Tetanus.

Apparatus:—Same as in previous experiment.

Perform this experiment, if possible, with the same muscle used in the previous experiment. Connect the plug-key with the inductorium, so as to obtain repeated induction shocks. Slow moving drum. Close the key and record a stretch of tracing. Individual tracing. What is the relation between this curve and that obtained by a single induction shock?

Exp. 11—Staircase and Fatigue.

Apparatus:—Same as in Exp. 9.

- (a) Set up induction coil for single shocks. Make a muscle preparation, place it in the holder and connect it with the secondary coil. Use a load from 30 to 60 grams, depending on size of muscle. Draw a base line clean around the drum. For the first dozen stimulations use the break shocks only (why?); after that both makes and breaks may be used.
- (b) With drum moving fairly slowly stimulate with break shocks at the rate of one per second (moderate strength). Notice the first few contractions. After 10 or 12 contractions stimulate with both make and break, continuing this until the muscle is entirely fatigued. Group tracing.
- (c) When this occurs, remove the weight, wash the muscle well with 0.7% NaCl and allow it to rest for 10 minutes. Replace the load and repeat. (b). When again fatigued, increase the strength of the current. Group tracing.
- (d) Remove the muscle and cut it into two parts. Press the cut surface of one piece on a strip of blue litmus paper, and that of the other piece on red litmus. Try this also with a fresh muscle. Explain the results. Source of the material responsible for this reaction? How formed? What normally becomes of it? Why did this not happen in the present experiment? What may this cause?

From this experiment fully discuss the subject of Work and Irritability.

Exp. 12.—Automatic Action. Smooth Muscle.

Apparatus:—Kymograph, time marker, key, stand, clamp holders, heart lever, muscle clamp, frog, adrenalin, $\frac{1}{2}\%$ barium chloride.

- (a) Draw a base line on a drum. Pith a frog; expose the stomach; cut a ring from the pyloric end of the stomach about 6 or 8 mm. wide. Insert into this ring two wire hooks, attaching one hook to the muscle clamp and the other to the writing lever. Place the writing lever on the drum about one inch above the base line (very slow speed); keep the muscle preparation moist. By signal magnet, record time in 5 seconds.
- (b) While the drum is moving very slowly, spontaneous contractions of the muscle may take place. Record a number of these. Group tracing. Is there any evidence of changes in muscle tone? Length of contraction and relaxation period? What property not possessed by the striated muscle is demonstrated?
- (c) When a stomach strip is recording its contractions apply a few drops of adrenalin (1:10,000). When this has taken effect apply some barium chloride.

Exp. 13—Cilia.

Apparatus:—Piece of cork, microscope, ether, frog, freezing mixture.

Pith brain of a frog, place it on its back and cut away the lower jaw. Wash the roof of the mouth with 0.7% NaCl. Place a small piece of cork near the angle of the jaw and watch the movement of the cork. Determine how long it takes to move one cm.

Moisten the cilia with salt solution having a temperature of 5 degrees, and again determine rate. Is the action of the cilia dependent on the central nervous system? Place the frog under a beaker and saturate the air under the beaker with ether. How are the cilia affected. Blow air over the cilia to remove the ether. Effect? With scissors remove a very small piece of the ciliated epithelium and examine with microscope. What physiological properties do the cilia exhibit? Where are cilia found in the body? What is the function of the cilia?

Exp. 14—Reflex Action.

Apparatus:—Inductarium, key, wire, electrodes, frog-board, frog.

Pith brain of a frog. Expose both sciatic nerves as follows:

Place frog, back upward, on frog board, slit open the skin on the thigh a little inside the median line. Carefully open the muscles without the use of a knife and expose the sciatic nerve. Take great care not to injure the blood vessels and do not stretch the nerve. Isolate and place a loose ligature under the nerve. Repeat for the other leg. With a weak interrupted current stimulate the left sciatic nerve. Effect? If none, increase strength of current. Now place two ligatures close together on the left sciatic and cut in between them. Leave a long end on each ligature so that you can handle the nerve by means of it. Stimulate the peripheral end. Result? Stimulate the central end. Result? How do you explain this? Can you prove this? Proceed to do so.

What structures are necessary for any reflex action? Which of these have you dealt with in this experiment? How could you demonstrate the necessity of the other?

Exp. 15—Reflex Action: Reflex Time.

Apparatus:—Frog, stand, hook, beakers, $\frac{1}{2}\%$ HCl, filter paper, 50% acetic acid.

Pith the brain of a frog (make sure of this by the lid reflex). Carefully notice the condition of the frog immediately after the pithing; pinch its toes. After 5 or 10 minutes pinch the toes again. Why did not the frog respond immediately after the operation? What is this condition called? Pass a hook through the jaw and suspend the frog.

- (a) What effect has pinching the toes of the foot? Place the long toe in $\frac{1}{2}\%$ HCl and note by watch how many seconds elapse before the foot is withdrawn. After the result has been noted bathe the foot thoroughly in tap water. Make three determinations and take the average. What is this period called? Why is there such a period?
- (b) On the flank place a small piece of filter paper (about 3 or 4 mm. square), which has been soaked in 50% acetic acid (shake off most of the acid). Notice the movement of the animal to remove the irritant. If the frog succeeds in removing it, wash the frog in tap water for at least one minute and let it rest for 4 minutes. Repeat the above, placing the paper in different positions (as on the thigh, etc.) and observe the behavior of the frog. Wash the frog thoroughly after each application.
- (c) Repeat (b) but while the paper is on the leg, pinch the toes of this leg. Result? How can you explain that the "spinal frog" was able to locate the pieces of paper?
- (d) Destroy the spinal cord of the frog and repeat (a). What does this teach? Make a diagram illustrating this. Could any other parts have been destroyed and the same results obtained as in destruction of the cord?

Exp. 16—Reduction of Nerve Conductivity.

Apparatus:—Induction coil, key, wire, plug-key, 1% cocaine, 1% novocain, $\frac{1}{2}\%$ HCl, frog, stovain.

- (a) Make a muscle-nerve preparation; use as long a nerve as possible. Determine the least strength of break induction shocks that will stimulate the nerve and cause the muscle to contract. This determines the threshold stimulus. Apply 1% cocaine to a short stretch of the nerve and after 5, 10, 15, etc., minutes again determine the threshold. Discuss your results.
- (b) Pith brain of a frog and determine the length of the reflex time (as in Exp. 15, a) using $\frac{1}{2}\%$ HCl. Make two trials and take average. After each determination wash the foot thoroughly with tap water. Now suspend one foot in 1% novocain and the other in 1% stovain and at 5 minute intervals again determine the reflex time for each foot. Explain the results.

Exp. 17—Reflexes in the Human Being.**(a) Tendinous or deep reflexes:**

1. The knee jerk. Sit on the table so that the leg from the knee down hangs free. Let another student strike the patellar ligament with the edge of a ruler. What is the result? Just previous to the striking of the ligament let the subject clench his fist. What effect does this have on the knee jerk? Explain. Of what diagnostic value may this reflex be?
2. The ankle jerk. Let the subject kneel on a chair, the feet hanging over the edge of the chair. With a ruler tap the tendon of Achilles and note the result.
3. The inferior maxillary reflex. Let the subject open his mouth. Place a lead pencil on the lower teeth; tap this with a ruler and observe the raising of the lower jaw. This does not always take place.

(b) Cutaneous or superficial reflexes.

4. Corneal reflex. Touch the cornea with a soft object.
5. Plantar reflex. Scratching the sole of the foot causes contraction of the toes.

(c) Organic reflexes.

6. Swallowing reflex. Empty the mouth of saliva by swallowing and immediately swallow again. Are you able to do so? When can you? What does this prove?
7. Pupil reflex. Close the eyes for two minutes; while facing a bright light open them and let another student examine them immediately.
8. Cilio-spinal reflex. Pinch the skin of the neck and note the dilation of the pupil.

PART THREE

Blood

Exp. 18—Hemolysis or Laking of Blood; Permeability of the Red Blood Corpuscle.

Apparatus:—Microscope, slides, covers, blood, distilled water, 0.7% NaCl solution, chloroform, ether, test tubes, $m/4$ NH_4Cl , $m/2$ NaCl, crystals of urea, KCl and dextrose.

- (a) Obtain some defibrinated blood and place a drop of it on a slide and test its transparency by trying to read print through it (hold the slide about one inch above the paper). Place another small drop on a slide and add five drops of distilled water to it; mix. To another drop add five drops of physiological salt solution. What difference in the transparency and color? How explained? What property of the salt solution prevents the laking of the blood?
- (b) The red blood cells are impermeable to NaCl; consequently, if the cells are placed in a hypertonic NaCl solution, they will lose water and become crenated. In a hypotonic NaCl solution the cells will absorb water and increase in size. Obtain a drop of blood on the point of a pin or knife; shake off most of it and make a small smear on a slide; add 3 or 4 drops of $m/2$ NaCl. Cover and study the changes in shape and size of the corpuscle. Illustrate your notes by diagrams.
- (c) Repeat (b) using $m/4$ NaCl solution.
- (d) Repeat (b) using $m/16$ NaCl solution. Describe and explain the results.
- (e) See whether an $m/8$ or $m/7$ NaCl has any effect. If not, why not? Save some of this solution for (g) and (h).
- (f) What is the osmotic pressure of $m/4$ NH_4Cl solution compared with that of $m/4$ NaCl? Determine the effect of $m/4$ NH_4Cl on the blood cell. Compare this with (c) and explain why this difference exists. Be sure to notice the immediate and the final result. Explain difference.
- (g) To 3 or 4 drops of the NaCl solution in (e) that had no visible effect on the cells, add a few urea crystals. How does this affect the osmotic pressure of the solution? With this solution repeat (b) and explain results.
- (h) Repeat (g) but use dextrose instead of urea. Also try KCl. What does this prove?
- (i) Hemolysis by chloroform, etc. Place a drop of blood in a clean dry test tube and add three drops of chloroform. Gently agitate for a little while. Pour contents on a slide and quickly note transparency. Repeat with ether. How do these reagents bring about this result? What property do these reagents have in common? What are reagents producing this result called?

Exp. 19—To Determine the Osmotic Pressure at Which the Hemoglobin is Driven from the Red Blood Corpuscle.

Apparatus:— $m/4$ NaCl solution, defibrinated blood, test tubes, graduated pipette, distilled water.

Take some $m/4$ NaCl solution; by diluting it with distilled water make about 10 c.c. of the following NaCl solutions: $m/6$, $m/8$, $m/9$, $m/9.5$, $m/10$, $m/10.5$, $m/11$, $m/12$. In the 9th test tube place 10 c.c. of pure water. Make these dilutions carefully and explain fully in your notes how you made them.

To each of the test tubes, add 5 drops of blood and shake the test tubes gently but thoroughly. Allow the test tubes to stand for $\frac{1}{2}$ hour (don't disturb); note results. Why this difference in the supernatant fluid? In what concentration do the corpuscles begin to lose their hemoglobin?

Exp. 20—The Specific Gravity of Blood.

Apparatus:—Blood lancet, absorbent cotton, urinometer, chloroform-benzol mixture, chloroform, benzol, alcohol.

To obtain blood proceed as follows:

Clean the palmar surface of the finger tip (or the lobe of the ear) with alcohol. Sterilize the lancet by means of alcohol. Puncture the skin with a quick and firm stab; the long axis of the cut must be diagonally across the finger top. If necessary press gently to start the flow; after this the blood must flow freely. Do not use blood squeezed out.

This experiment must be worked as quickly as possible. Put the mixture of chloroform and benzol of about 1058 sp.gr. (from large bottle) into a glass cylinder. Be sure this is clean and dry; care must be taken to get no water in the reagents. Obtain a drop of blood as directed and transfer it to the mixture. If it sinks add chloroform drop by drop; if it rises, add benzol; after each addition of chloroform or benzol place your hand tightly over the cylinder and gently invert the tube three or four times to insure thorough mixing. Repeat this process till the drop remains suspended wherever it is placed. When this occurs, immediately (why?) take the sp. gr. of the mixture. Give reasons for procedure? What is the normal sp. gr.? How does this compare with the sp. gr. of milk, lymph or urine? What causes the high sp. gr. of blood? What is indicated if the sp. gr. is low? Make a sketch of the apparatus.

N. B.—At the close of the experiment filter the mixture and restore it to the mixture bottle. Do not waste any of the reagents. Do not let the bottles remain uncorked.

1.040=40 per cent Hb.

1.050=65 per cent Hb.

1.055=75 per cent Hb.

1.060=95 per cent Hb.

Exp. 21—Determination of the Number of Red Blood Corpuscles.

Apparatus:—Hemocytometer, absorbent cotton, 3% NaCl, microscope, lancet, alcohol-ether.

1. Obtain a large drop of blood as directed in Exp. 20.
2. Take the large tube and suck up the blood till it reaches the mark 1. Dry the tip of the pipette. If the 1 mark should be passed, touch the point of pipette with filter paper until the column of blood stands at 1. Immediately suck up 3% NaCl solution till the mixture reaches the 101 mark. Rotate the pipette while filling it. Make these measurements very carefully. If the 101 mark is passed, the tube must be cleaned as directed below, and the work started afresh.
3. Close the end of pipette with the finger and shake the glass bead in the bulb of the tube, thoroughly mixing the blood and the solution. How many times has the blood been diluted?
4. Blow out 5 or 6 drops. Why? Wipe the end of the pipette and let a small drop form at the end.
5. Touch this to the ruled disk and allow a small drop to flow upon the disk of the counting slide; cover quickly, press the edges of the cover down gently. Do not slide the cover over the drop. No blood should run over into the moat. Let the corpuscles settle for 4 or 5 min. If blood runs into the moat, clean the slide as directed below and use a smaller drop.
6. Place it on the microscope stage and notice the lines ruled on the glass (high power). Be very careful in focusing. Count the number of RBC in at least 20 cells and take the average. The size of the cell is $1/20 \times 1/20 \times 1/10$ millimeter. How many RBC in one cu. mm.? (Remember the blood has been diluted.) What per cent is this of the normal?
7. Sketch the apparatus.

CAUTION:—Never let the blood coagulate in the tube. If the blood does not form a solid column in the tube, the tube has not been properly filled and you must blow the blood out immediately and clean the tube first with distilled water till *all the blood has been removed*, then with alcohol and lastly with ether. Let this last run out and pass compressed air through the tube until it is dry. If thoroughly dry, the glass bead will not stick to the sides of the tube. In case the blood can not be removed by means of distilled water, use water solution of hydrogen peroxide; if this fails, call the assistant. Clean the slide and cover with distilled water and absorbent cotton; never with alcohol or ether. Do not scratch the slide and keep it out of direct sunlight.

Exp. 22—Determination of White Blood Corpuscles.

Apparatus:—Same as in Exp. 21.

Follow the directions in Exp. 21 but use the other tube, marked 1 and 11, and use the methyl-violet diluting fluid. This colors the white cells only. Hold the tube horizontally. Count the white cells in 60 squares. Average? Number per cu. mm.? What is the normal number? Ratio of red to white corpuseles?

Exp. 23—Sahli's Hemoglobinometer.

Apparatus:—Sahli's hemoglobinometer, lancet, cotton, n/10 HCl.

Note the following parts of the apparatus:

1. A capillary measuring tube with a mouthpiece.
2. The standard color tube.

hemoglobin stated? What variations in the amount of Hb?

Fill the mixing tube up to about the 10 mark with n/10 HCl. Obtain a good sized drop of blood; suck the blood up into the capillary tube to the 20 cu. mm. mark. Blow the blood into the acid in the mixing tube; suck up a little of the acid into the tube and return to the mixture. In this manner the capillary tube is rinsed and the acid and blood are thoroughly mixed.

After about a minute the mixture will be dark brown and clear. Add a small quantity of water, shake and compare the color with the standard by holding the tubes between your eye and the light. If necessary add more water and again shake well. Towards the end the water must be added drop by drop and after each addition the tube must be shaken and its color compared with the standard. When the two tubes have the same color, read the scale on the side of the tube. Make at least two determinations for the same (Why?) person's blood. What is the normal amount of hemoglobin? How is the amount of hemoglobin stated? What variations in the amount of Hb?

Exp. 24—Blood Quotient.

From experiment 21 and 23 calculate the blood quotient according to following formula: Per cent Hb divided by per cent number RBC equals the blood quotient. What is meant by the blood quotient? Object of determining it?

Exp. 25—Hemoglobin and its Derivatives.

Apparatus:—Test tubes, defibrinated blood, hydrogen peroxide, ammonium sulphide, sodium nitrite, guinea-pig. Canada balsam, amyl alcohol, slide and coverslip, microscope.

- (a) In a test tube place about 10 c. c. of defibrinated blood. Shake it well with air. Color? What kind of blood is this? What compound is formed? Divide this blood into two equal parts.
- (b) To one part of (a) add a drop or two of ammonium sulphide and gently mix. Compare color with (a). Why different? What kind of blood is formed in (b)? What is the color due to?
- (c) To (b) add a few drops of hydrogen peroxide; shake tube. Account for change in color. Where in our body does (a) occur? Where does the change illustrated in (b) take place? What brings about this change? What is its object? What valuable property of Hb is illustrated in (a), (b), and (c)?
- (d) Slowly pass CO (illuminating gas) through about 10 c. c. of defibrinated blood to which you have added one or two c. c. of amyl alcohol (to prevent foaming). After 10 minutes compare its color with tube (a). To one-half of this blood add a few drops of ammonium sulphide. Result? Compare with (b). Why different? What is the result of the inhalation of CO? Why?
- (e) Dilute one c. c. of defibrinated blood with 10 c. c. water. Divide into two parts. To the one part add a few drops of sodium nitrite (or amyl nitrite). Observe the change in color. The compound formed is methemoglobin. What is said to be the important difference between this and oxyhemoglobin? What is one of the results if the change illustrated in this experiment takes place in the animal body? Why?
- (f) On a slide place a drop of guinea-pig's blood and on this a drop of Canada balsam. Cover and examine for crystals of Hb. Draw.

Exp. 26—Guaiacum Test for Blood.

Apparatus:—Hydrogen peroxide, guaiacum tincture, potato, blood, graduate, test tubes, burner.

- (a) Place two or three drops of defibrinated blood in a test tube. Add 2 drops of guaiacum and a little hydrogen peroxide. The blood decomposes the H_2O_2 and the guaiacum is oxidized, giving rise to a blue color. Next drop a few drops of guaiacum and H_2O_2 on a piece of potato (immediately beneath the skin). Boil a small piece of potato for 3 or 4 minutes and make the test. Try the same with blood.

In the potato and other substances this action is brought about by enzymes called oxydases. These enzymes also exist in the blood, but in addition to this the Hb (or its iron-containing derivatives) has the power to split up the peroxide. In how far is this a good test for blood? How can it be rendered more accurate?

- (b) Thoroughly clean 6 test tubes. Dilute one volume of blood with 800 parts water (1 drop of blood plus 40 c. c. water), and from this make dilutions with 1600, 3200, 9600, and 28,800 parts water. Place one c. c. of each in one of the test tubes and in the sixth tube place water. To each add a few drops of benzidin dissolved in glacial acetic acid and a drop or two of hydrogen peroxide. Discuss the value of this test.

Exp. 27—Hemin or Teichmann's Crystals.

Apparatus:—Microscope, slides, covers, dried blood, glacial acetic acid, Bunsen burner.

Place a few small scrapings of dried blood on a slide or carefully evaporate some fresh blood to dryness. As this is fresh blood no NaCl needs to be added. Triturate. Add 2 or 3 drops of glacial acetic acid and cover with coverslip. Warm one end of this slide carefully over a small flame till bubbles begin to appear. Take out of flame, add another drop of acid and warm again. When cool examine with microscope, high power. Draw some of the crystals. Color? Give three reasons why this is a good test for blood. What disadvantage?

Exp. 28—Defibrinating the Blood; Fibrin; Serum.

Apparatus:—Test tubes, artery cannula, artery clips, wood for whipping blood, dog, 4 inches of rubber tubing, slides, covers, microscope, 25% acetic acid.

- (a) To a dog administer by means of a stomach tube 0.25 gram chloretone per kilogram of body weight. When the animal is under the influence of the drug, place it on a dog-board. If necessary cut the hair from the region over the trachea. Make a longitudinal incision through the skin and fascia over the trachea. By blunt dissection separate the mesial borders of the sternohyoid muscles, thereby exposing the trachea. Along side of and a little dorsal to it you will find the carotid artery. Feel for the pulsation. Expose a stretch of this artery and clean it carefully from all fascia. Place a ligature at the distal end of this part and an artery clip at the proximal end. Between these two make a V-shaped incision with scissors (point towards the heart) and insert a cannula, securing it in the artery by means of a ligature. Open the artery clip, catching some blood in a dish. Let one of the students immediately whip this blood with a roughened piece of wood. The fibrin collects on the wood; retain both the liquid and the fibrin.
- (b) Place a drop of blood issuing from the cannula on a slide and place this in a moist chamber. After a few minutes examine this with the microscope (low power) for threads of fibrin and the clumping of the corpuscles. Continue to observe it for at least 15 minutes.
- (c) Let a little blood from the artery flow into a test tube containing some hirudin. Set it aside and note the result.
- (d) Catch about 5 c.c. of blood in each of three test tubes:—(a) a vaselined tube, (b) a tube containing a pinch of sand, and (c) an untreated tube. Record the time the blood was placed in these tubes. By gently tilting the tubes at intervals of one or one half minutes, determine the coagulation time for these three samples of blood. Explain any difference. Let tube (c) stand for 24 hours and describe its appearance. What is the fluid formed called?
- (e) Pour some of the defibrinated blood obtained in (a) either into a test tube and let it stand to settle for at least 24 hours, or into centrifuge tubes and centrifuge until a clear serum is obtained. Note the relative amount of corpuscles and serum.
- (f) By means of a pipette carefully remove about 5 c.c. of the serum without disturbing the red corpuscles. To the serum add 2 drops of 25% acetic acid and coagulate the protein by boiling. What proteins are found in serum? In blood?
- (g) Take a small piece of the fibrin obtained in (a) and wash it thoroughly in water, kneading it meanwhile. Describe the characteristics of fibrin. Solubility in water? Place a shred of fibrin in a little water and make Millon test. To what class of bodies does fibrin belong? Its origin? Its use?

Exp. 29—Preventing Coagulation.

Apparatus:—Oxalate blood. Salt-plasma, 2% CaCl_2 , test tubes, ice, water bath, thermometer.

- (a) The instructor has caught some fresh blood in a dish containing a little sodium oxalate solution. Obtain 15 c.c. of this oxalate blood. Condition: To 5 c.c. add 7 drops of 2% CaCl_2 and to another 5 c.c. add 20 drops. Place in the water bath at 40 deg. C. and by your watch note in how many seconds or minutes clotting begins. When the blood is well clotted the clot adheres closely to the walls of the tube. Let the tube stand for at least 24 hours and notice a solid mass suspended in a liquid. What are these two parts called? Color of the liquid? What part does calcium play in coagulation? Why is oxalate blood liquid? Place another 5 cc. of oxalate blood on ice and when it has a temperature of 5 degrees C. add the 7 drops of CaCl_2 and determine length of coagulation time.
- (b) To another quantity of fresh blood an equal volume of half saturated Na_2SO_4 solution has been added. This has been allowed to stand for some time and the corpuscles have settled. Notice the more or less clear plasma. Dilute about 5 c.c. of this salt-plasma with 25 c.c. distilled water. Place half of this diluted salt-plasma in a test tube containing a piece of blood clot or a few drops of serum and set it in warm water bath. Also place the remainder in the bath and note length of coagulation time. Why is this shorter in the first tube? How could you differentiate plasma from serum?

PART FOUR

Circulation

Exp. 30—Anatomy of the Mammalian Heart.

- (a) Note the following structures:—
1. The right and left auricles.
 2. The right and left ventricles.
 3. The aorta and the pulmonary artery.
 4. The superior and inferior vena cava and the four pulmonary veins.
 5. The coronary vessels.
- (b) Cut open the two auricles and note:—
1. Thickness of auricular walls.
 2. Absence of valves at mouth of veins.
 3. The septum between the two auricles.
 4. The auriculo-ventricular orifice.
- (c) Make a series of cross sections of the ventricles, beginning at the apex and working toward the base until both cavities and the auriculo-ventricular valves are brought into view. Note:—
1. Thickness of walls of the two ventricles. Why is this difference necessary?
 2. The columnae carneae. The chordae tendineae and their attachment to the valves.
 3. The bicuspid and tricuspid valves.
 4. The openings into the aorta and into the pulmonary artery.
 5. The semilunar valves.
- (d) Securely tie a funnel into the aorta or pulmonary artery and pour water into it. Notice the closure of the valves, preventing the escape of the water into the ventricles. From below push the valves open.
- (e) Slit open the pulmonary artery and the aorta so as to expose the valves. Note: —
1. The number and shape of the segments.
 2. The sinuses of Valsalva. Their function?
 3. The mouth of the coronary artery.
 4. Compare the thickness and elasticity of the walls of the arteries and veins. Why is this difference of great consequence?

Exp. 31—Anatomy of Frog's Heart.

Apparatus:—Frog board, frog.

Pith the brain of a frog. Fasten the frog to frog board, back down. Cut through the skin over the thorax and then cut through the chest wall, thereby exposing the heart with as little loss of blood as possible. Keep the heart moist with Ringer's solution. Observe and describe:—

- (a) The pericardium. Notice its thinness, strength and inelasticity. What are its functions? Slit it open.
- (b) The two auricles.
- (c) One ventricle. Compare its wall with that of auricles. Notice the auriculo-ventricular groove.
- (d) Bulbus arteriosus springing from the ventricle and dividing into two aortae.
- (e) On dorsal side is the sinus venosus emptying into the right auricle. To see this tilt the apex of the ventricle upward and forward. A white crescent-like structure will be seen; this separates the auricles from the sinus.
- (f) The two superior and one inferior venae cavae emptying into the sinus. Draw a diagrammatic sketch of the frog's heart.

(e) SB. closed; obtain about 100 mm. Hg. pressure.

1. Notice the pulse; its characteristics, such as frequency, hard or soft, slow (*pulsus tardus*) or quick (*pulsus celer*). A quick pulse is not necessarily a frequent pulse, but one that reaches its maximum quickly—one might say a sharp, kicking pulse. What is the pulse?
2. Now partially open SB. so as to obtain with the same rate of pump, a pressure of about 30 mm., and repeat (1). Can you account for the difference?
3. Close SB. Pump so as to have 30 mm. pressure and repeat (1). Is there a pulse in the veins in (1), (2) or (3)? Under what conditions in the living body is there a venous pulse?

(f) Having SB. closed, obtain about 100 mm. Hg. pressure. Slow the stroke to about 20 per minute, and notice the valves. By the gush of water from beneath the rubber flap covering the hole in the metal tube you can judge of the opening of the valve. With respect to the systole and diastole, determine the opening and closing of both valves. Do they both open at the same time? Reason? As to their closing?

(g) With SB. closed, obtain fairly high Art. P. Maintaining the same number of strokes of even force, gradually open the SB. and note:

1. Effect on Art. P.? Is the rise synchronous with the systole of the pump? Why?
2. What condition of the vascular system does this represent? The stimulation of what nerve has this effect?

Exp. 33—Graphic Record of Heart-Beat.

Apparatus:—Frog board, kymograph, frog, heart lever, stand, clamps, signal magnet, wire, key, a hook, tuning fork, thread.

- (a) Pith the brain of a frog; inject a couple of drops of curare into the dorsal lymph sac. When the curare has taken effect, place frog on frog board and expose the heart and slit open the pericardium with as little loss of blood as possible. Pass a fine hook (bent pin) through the tip of apex so as not to puncture the ventricle, and by means of thread connect this hook with the short arm of the heart lever. When the heart contracts, the auricle first pulls the lever up a slight distance, then the ventricle contraction raises it still further. It may be possible to detect the contraction of the sinus venosus. The lever must be balanced delicately by means of counterweight, so that the heart is stretched to a very slight extent. Keep the heart moist. Let the writing point come in contact with the drum so as to make a mark when the lever is raised and lowered, but there must not be much friction. Place the point of the signal magnet (recording seconds) directly below the point of the heart lever. Record a stretch of heart beat (about 2 or 3 inches). Individual tracing. Label: Graphic Record of Frog's Heart.
- (b) Use same speed of kymograph. Cool some Ringer solution to 5°C and by means of a pipette cool the heart. When you have reason to believe the heart is well cooled take a tracing (Group tracing). Next use Ringer solution at 12°C and then at 25°C. Compare the rates at the various temperatures.
- (c) Fast kymograph. Instead of the signal magnet use the tuning fork. Obtain a tracing of two heart beats with the tuning fork tracing. Individual tracing; same legend as above. When the tracing is dry, draw perpendiculars from the characteristic points of the heart tracing to the tuning fork tracing. This will enable you to calculate the length of the various phases of the cardiac cycle. Calculate:—
1. Rate of heart beat per minute.
 2. In hundredths of a second:

Auricular systole.....	}	Total.....
Auricular diastole	}	
Ventricular systole.....	}	Total.....
Ventricular diastole.....	}	

3. Ventricle works.....per cent of the time.

Ventricle rests.....per cent of the time.

**Exp. 34—Properties of the Heart Muscle. Effect of Previous Stimulation;
Stair-case Phenomenon.**

Apparatus:—Frog board, frog, thread, aneurism needle, induction coil, wire, key, electrodes.

To study certain properties of the cardiac muscle, it is often necessary to have a quiescent heart. This can be obtained by means of the First ligature of Stannius.

- (a) Pith brain and cord of a frog; expose heart and remove pericardium from heart and aortae. Pass a ligature beneath the bulbus arteriosus and bring it forward and tie it between the auricles and the sinus venosus, the sinus will continue to beat, but the rest of the heart will be quiet. If these results are not obtained apply another ligature.
- (b) Stimulate the ventricle with a pin or a single induction shock. What properties of the ventricular muscle are here demonstrated?

The cessation of beat in the auricles and ventricle by the first Stannius ligature is generally attributed to the stimulation of the inhibitory mechanism in the sinus. Others hold that the ligature merely destroys the conductivity and the impulse originating in the sinus cannot pass down the rest of the heart.

- (c) Set up recording apparatus as in Exp. 33 (a). Slow drum. Stimulate the quiet heart with a medium strength break induction shock (short circuit the make). After about 5 seconds repeat and so on until you have recorded about 10 contractions, using the same strength of break induction shocks. Discuss your results. Group tracing. Label the tracing "Staircase Phenomenon of Heart."

Exp. 35—Properties of Heart Muscle. “All or Nothing.”

Apparatus:—As in Exp. 34.

If possible use the frog of Exp. 34, otherwise repeat Exp. 34 (a). Record by suspension method. Stimulate the quiet ventricle with the weakest possible single break induction shock (short circuit the make). If a contraction occurs, record it on the stationary drum; after each contraction move the drum about $\frac{1}{2}$ inch. After one minute (not sooner) move secondary coil 1 cm. closer to the primary and repeat the above (use break shocks only). Record contraction (if any) as before. After another minute rest repeat with a little stronger current. Continue this until full strength of current is used. Under each line of the tracing state distance between primary and secondary coils. How do the results obtained compare with those obtained with the skeletal muscle (Exp. 9)? Why this difference? Why is it necessary to wait about one minute between stimulations? Group tracing. Label your tracing “All or nothing of frog’s heart.”

Exp. 36—Properties of Heart Muscle. Extra-Systole. Compensatory Pause. Refractory Period.

Apparatus:—Key, induction coil, kymograph, frog board, stand, clamps, electrodes, signal magnet.

Place the signal magnet in the primary circuit so as to indicate point of stimulation. The point of the signal must be directly above or below the point of the heart lever; this is very essential to the success of the experiment. Expose the heart of a pithed frog and obtain a tracing, as in Exp. 33, on a medium speed drum. Stimulate the ventricle with a single break shock only, short circuiting the make shock. Note the resulting extra-contraction (if any) and the phase of the heart when the stimulation was thrown in. Repeat this stimulating about five or six times per minute. Some of the stimuli will fall during the systole, others during diastole. Note effect. When does the heart respond to the stimulation and when does it not? What is this last period called? When an extra-contraction takes place, notice the following period of rest; how does it compare with the normal? What is this called?

Group tracing. Label your tracing “Extra-systole, compensatory pause and refractory period.”

Exp. 37—Transmission of Cardiac Excitation. The Block.

Apparatus: Frog board, Gaskell's clamp, a large frog.

- (a) Notice the orderly sequence in the beating heart. Does the ventricle beat as often as the auricle? Which is in systole first? The impulse therefore travels in what direction? Notice the pause between the auricular and the ventricular systole. What does this pause indicate as to the tissue uniting the auricle and the ventricle? How can we explain the fact that the impulse begins in the sinus?
- (b) Now place Gaskell's clamp at the auriculo-ventricular groove. Turn the screw so that the rubber edge just touches the heart. Count the auricular and the ventricular contractions. If normal, turn the screw just a trifle more and again observe the effect. Continue this process very slowly until there is a difference in the auricular and ventricular rate of beat. Record four readings of the rates of the two cavities with gradually increasing pressure.

Note:—Let one student count the auricular and another the ventricular beat at the same time. Explanation? What condition is supposed to produce the same result in man?

If you can succeed in stopping the ventricle altogether, touch the quiescent ventricle with the point of a knife. Result? What property does the ventricle possess?

- (c) Release the clamp and place it on the ventricle midway between base and apex, using enough pressure to cause the apex to be quiescent when the clamp is removed. When this has been obtained close the aorta with forceps and observe the apex. Explain the results.
- (d) Pith a frog. Expose the heart and make 4 or 5 interdigitating sections across the ventricle. Note the contraction of the strips. What does this demonstrate? Cut the ventricle from the auricle. Result? Explanation? Stimulate the apex with a single induction shock. In what direction did the impulse travel?

Exp. 38—Influence of Temperature on Heart.

Apparatus:—Frog, freezing mixture, glass dish, test tubes, thermometer.

Count the rate per minute of the exposed frog heart. Excise the heart (See Exp. 32, h). Place the heart in a dish filled with Ringer's solution. Does it continue to beat? What does this prove as to the relation between the heart beat and the central nervous system? Take the temperature of the solution and determine the rate of the beat. Now transfer the heart to a test tube containing about 5 c.c. of Ringer's solution, having a temperature of about zero. When the heart has acquired this temperature, determine rate of heart. As the tube stands on the desk, the temperature will gradually rise. Determine the rate for every three degrees rise. When the room temperature is reached, carefully add warm Ringer's solution and proceed in the same manner till 35 degrees C has been reached. Besides the changes in the rate of the beat, are there any other changes such as strength and rapidity of contraction and relaxation; rhythm? As the heart beat is increased, is the shortening of the diastole the same as that of the systole?

Plot a curve on cross-section paper, using the degrees as abscissae and the rates as ordinates.

Exp. 39—Action of Chloroform and Ether.

Apparatus:—Chloroform, ether, graduated pipette, two frogs, test tubes.

Excise the hearts of two small frogs of the same size; be sure to include the sinus venosus. Place in a dish with Ringer's solution, and by watch obtain rate per minute. After one or two minutes transfer the one to a test tube containing 15 c.c. Ringer's solution (accurately measured) and 3 drops of chloroform; cork. Transfer the second heart to 15 c.c. Ringer's solution plus 3 drops of ether; cork. Obtain rate after 1, 2, 4, 7, 10, 15, 20 and 25 minutes and note exactly how long both hearts continue beating. In what phase do they stop? Discuss the relative potency of these two drugs.

On co-ordinate paper construct two curves, in colors, showing the rate of beat before and after the action of the drugs.

Exp. 40—Cardiogram; Cardiac Impulse.

Apparatus:—Kymograph, cardiograph, time marker, wire, key, tambour.

- (a) Locate the cardiac impulse of a fellow student. Place the button of the cardiograph over the cardiac impulse. The cardiograph is connected by means of a tube with the recording tambour. In this tube is a T-tube which must be closed when everything is in readiness for a tracing. Let the pen of the tambour write on a medium speed drum. Also record time in seconds. Group tracing. Study the tracing obtained, consulting text-books on the subject.
- (b) Note the changes in the position and strength of the apex beat as the subject lies on his left or right side and as he stands. What do you recall about its position in pathological conditions? What about the force of the apex beat? Its origin?

Exp. 41—Heart Sounds.

Apparatus:—Stethoscope.

This experiment is to be performed in a quiet room. Locate cardiac impulse in a fellow student. Apply the stethoscope to this point and note the two cardiac sounds. Note differences in pitch, loudness and duration of these sounds. Feel of the radial pulse and note synchronism with the first or second sound. Observe the pause following the first sound and compare with that following the second sound. While listening to the sounds feel with the finger for the apex beat and note when this occurs with respect to the sounds. What are the causes of the two heart sounds?

Place stethoscope to the second right costal cartilage; here the second sound is best heard. Why? The sound caused by the pulmonary valve is best heard over the second left costal cartilage.

Exp. 42—The Pulse.

This experiment is to be made at home. Count the rate of the radial pulse under the following conditions:

- After lying down for 15 minutes.....
- After sitting for 5 minutes.....
- After standing for 5 minutes.....
- Immediately after violent exercise.....
- During prolonged and deep inspiration.....
- During prolonged and deep expiration.....
- Before rising in the morning.....
- 15 minutes after rising.....
- At noon
- Before principal meal.....
- 10 or 15 minutes after meal.....
- 6 P. M.....
- Just before retiring.....
- On cross-section paper plot these variations.

Exp. 43—The Pulse Tracing. The Sphygmograph.

Apparatus:—Sphygmograph, kymograph, paper.

Cut 5 or 6 papers to fit the sphygmograph; smoke them (or use the kymograph.)

Adjust the button of the sphymograph over the radial artery with sufficient pressure to flatten the artery. Notice the small wheel connected with an eccentric by which the pressure can be altered. Diminish the pressure until a maximum excursion of the pen is obtained; now let the paper run through. Observe the primary and dierotic wave and state probable cause of each. Is the tracing normal? In what conditions is the dierotic wave small? Group tracing.

Exp. 44—Blood Pressure in Man. The Sphygmomanometer.

Apparatus:—The sphygmomanometer, stethoscope.

- (a) This experiment is to be performed in a quiet room. Fasten the rubber bag to the upper arm by means of the strip of cloth. Place the bell of the stethoscope over the bifurcation of the brachial into the radial and ulnar arteries (just below the bend of the elbow). Notice that no sound is heard. Inflate the rubber bag; if the pressure is high enough, no sound will be heard. Now, by means of the valve, very gradually reduce the pressure. At a certain point a sound is heard; this indicates that the blood pressure is capable of overcoming the outside pressure, and that blood is being sent through the artery. The highest pressure at which the sound can be heard is the systolic pressure. Next reduce the pressure still more; the lowest pressure at which the sound can still be heard is the diastolic pressure. To avoid congestion the pressure should not be left very long on the arm. Record three systolic and three diastolic pressures and take average.
- (b) Calculate the pulse pressure and the mean pressure.
- (c) Influence of position of body. Take systolic pressure while standing and sitting. Next lie down on the table and determine it after 5 minutes. Why does this difference exist?
- (d) Determine the effect of amyl nitrite on blood pressure (use one pearl). Explain.

	Systolic Pressure	Diastolic Pressure	Pulse Pressure	Mean Pressure
Reclining				
Sitting				
Standing				
After inhaling Amyl nitrite				

Exp. 45—Venous Blood Pressure.

Hold your hand down and note the veins on the back of the hand. Raise the hand high above your head and notice the collapsing of the veins. Why?

Now place your hand at the level of your heart and then slowly raise the hand until a height is reached where they collapse. Measure the distance (in millimeters) between these two points. What does this indicate?

Exp. 46—Capillary Circulation.

Apparatus:—Web-board, microscope, pins, frog, 0.7% NaCl.

- (a) Pith the brain of a frog. Inject into the dorsal lymph sac a couple of drops of a 1% curare solution. Object? Fasten the frog to the web-board. In this experiment either the tongue or the web may be used. The tongue of the frog is fastened at the anterior end only. Pull the tongue forward and pin the bifurcated end over the hole at the end of the board. Do not stretch the tongue or web too tightly as this may stop circulation; if this should happen, release the organ somewhat. Keep it moist continually with salt solution. Examine with low power of microscope and determine the following:—
- (b) Find a small artery. How can you tell the difference between this and a vein (direction of flow)?
- (c) Nature of the flow in an artery? Why?
- (d) Observe a small vein. Character of flow. Why?
- (e) Compare velocity of flow in capillaries with that in arteries or veins. Explain difference.
- (f) View with high power. If necessary place a small piece of coverslip on the tongue. If the flow of the blood is too fast, apply gentle pressure at the end of the tongue. Find a capillary and notice its width as compared with the diameter of the red blood corpuscle (R. B. C.)
- (g) Shape of R. B. C. Of the nucleus.
- (h) Have you found evidence of the elasticity of R. B. C.?
- (i) Character of the flow in a capillary? Why?
- (j) Observe with high power a slightly larger vessel. Can you distinguish between the red and white B. C.?
- (k) Do you notice any difference in the location of the red and white B. C.? Why? Do they move equally fast? Reason?
- (l) Color of the peripheral plasma free from R. B. C.?
- (m) Notice the sudden appearance and disappearance of capillaries. Explain.

Exp. 47—Hemodynamics.

Apparatus:—Artificial circulation scheme, distilled water.

In this system the pump represents the left ventricle; the two valves, the mitral and aortic valve respectively; the resistance of the wood, the capillaries; the tubes between the pump and the resistance, the arteries; those on the distal side of the resistance, the veins. The opening of the side branch substitutes a wide branch for the narrow one; this is equivalent to a dilation of the vessels. The mercury manometers measure the pressure; the one, the arterial pressure (Art. P); the other, the venous pressure (Ven. P).

Fill the reservoir with distilled water and pump the apparatus full, adding more water as needed; be careful not to force the mercury out of the arterial manometer. Make a diagram of the apparatus, naming the various parts.

(a) With side branch (SB.) open work the pump 10 strokes in $1\frac{1}{2}$ minute.

1. When does most of the water escape, during or between the strokes? Why?
2. Is the venous outflow continuous or intermittent?
3. Note arterial pressure (Art. P). Record its height during systole and diastole in mm. of Hg. What is the average pressure?
4. Gradually increase the number of strokes. How are 1, 2 and 3 affected?

(b) Close SB. Pump about 60 per minute.

1. Nature of outflow from the veins? Why?
2. Arterial P. Constant? Nature and extent of variation as compared with (a) 3?
3. Compare Ven. and Art. pressure. Why these differences?
4. Feel for pulse in artery and vein.
5. Feel of the arteries and notice fullness and hardness.

(c) Open SB. Pump 20 per minute.

1. Nature of venous outflow?
2. How could a uniform venous flow be obtained without closing SB.? Try it. Would this be practicable in the living animal?
3. From (b) and (c), what two factors are necessary to produce a constant venous flow? What advantage in a constant flow?

(d) While a good pressure is up and SB. closed, cease pumping. This corresponds to vagus inhibition. Determine the following:

1. Does Art. P. fall to zero immediately? Why?
2. Notice and describe venous outflow. Give reason for this continued flow.
3. What other benefit of elastic arteries is here suggested?

(e) SB. closed; obtain about 100 mm. Hg. pressure.

1. Notice the pulse; its characteristics, such as frequency, hard or soft, slow (*pulsus tardus*) or quick (*pulsus celer*). A quick pulse is not necessarily a frequent pulse, but one that reaches its maximum quickly—one might say a sharp, kicking pulse. What is the pulse?
2. Now partially open SB. so as to obtain with the same rate of pump, a pressure of about 30 mm., and repeat (1). Can you account for the difference?
3. Close SB. Pump so as to have 30 mm. pressure and repeat (1). Is there a pulse in the veins in (1), (2) or (3)? Under what conditions in the living body is there a venous pulse?

(f) Having SB. closed, obtain about 100 mm. Hg. pressure. Slow the stroke to about 20 per minute, and notice the valves. By the gush of water from beneath the rubber flap covering the hole in the metal tube you can judge of the opening of the valve. With respect to the systole and diastole, determine the opening and closing of both valves. Do they both open at the same time? Reason? As to their closing?

(g) With SB. closed, obtain fairly high Art. P. Maintaining the same number of strokes of even force, gradually open the SB. and note:

1. Effect on Art. P.? Is the rise synchronous with the systole of the pump? Why?
2. What condition of the vascular system does this represent? The stimulation of what nerve has this effect?

Exp. 48—Cardiac Inhibition, Pilocarpin and Atropin.

Apparatus:—Turtle, turtle-board, bone saw, kymograph, heart lever, induction coil, key, wire, signal magnet.

- (a) Destroy the brain of a turtle; fasten the turtle, back down, on board and with saw cut through the connections between the carapace and the plastron. Finish the removal of the plastron by cutting through the skin and other structures close to the plastron. Stretch the neck and hold the head in position by driving a nail through the jaw and into the board. Open the pericardium. Cut through the skin on the ventral side of the neck, separate the muscles and find the right vagus nerve. Isolate it and pass a loose thread under it.
- (b) Meanwhile let another student smoke a drum. Also arrange for repeated induction shocks. Place the signal magnet in the primary circuit and let the point of its pen write directly beneath the point of the heart pen. This will indicate time of stimulation.
- (c) When you think that you have found the vagus, verify this by stimulating it for a few seconds and note effect on heart.
- (d) Use suspension method as in Exp. 33. When you have recorded about 6 or 7 beats on a medium speed drum and the two pens are in proper position, stimulate the vagus for 5 or 10 seconds and obtain inhibition. If none results, gradually increase the strength of the current. When proper strength of current has been found, make a tracing showing escape from inhibition, keeping the current on for a longer time. Does inhibition occur immediately after the beginning of stimulation? What is the period elapsing between these two events called? Is the beat, if any, during this period the same as before stimulation? In which phase is the heart arrested? Notice and discuss the extent of the dilation during inhibition. How do the beats after stimulation compare with those before? Individual tracing.
- (e) Stimulate with repeated induction shocks the sino-auricular region and make tracing (group) of result. What structures were here stimulated?
- (f) Take 6 or 7 normal heart beats on the drum. Stop the drum. By means of a pipette apply a little 1% pilocarpin hydrochlorate to the heart. When a decided effect has taken place, record 4 or 5 beats. Stop the drum. Now apply some $\frac{1}{2}\%$ atropin sulphate and when it has taken effect record a few beats. Now stimulate first the vagus and then the sino-auricular region. Result? Explain. Group tracing.

Exp. 49—Reflex Inhibition of the Heart. Goltz's Tapping Experiment.

Apparatus:—Frog board, induction coil, key, frog.

- (a) Destroy the cerebrum of a frog by cutting off the head just back of the eyes. This leaves the medulla uninjured. By as small an incision as possible expose the heart so that its rate can be observed. Do not pinch the legs of the frog too tightly down on the board as this may interfere with the experiment. Observe the condition of the heart (color and rate). Tap the abdominal wall in the region of the stomach with the handle of a scalpel at the rate of 50 or more per minute. Observe the reaction upon the heart. If heart stops, in which phase? Note the heart beats immediately succeeding the inhibition. What is the condition of the heart? Is it normal? What nerves and what parts of the central nervous system are necessary for this reflex act?
- (b) Cover the heart with moist filter paper. Expose a long stretch of the sciatic nerve, as directed in Exp. 50 (a). Do not stretch the nerve. Place two ligatures on it, close together, and cut the nerve between the ligatures. Stimulate the peripheral end with repeated induction shocks. Any effect on the heart? Stimulate the central end. Result? What kind of fibres did you stimulate?
- (c) Discard the platinum electrodes and use ordinary wires. Place one electrode on lower part of the spinal column and the other on the cut surface of the brain (be careful not to injure the medulla). Stimulate with medium strength repeated induction shocks. Explain results. While heart is in inhibition, prove that it is irritable by pricking it with a blunt pin.
- (d) Tilt ventricle upward and forward. Place electrodes on the under surface of heart (on sinus venosus) and stimulate. The inhibition frequently takes place after the electrodes are removed.
- (e) Now destroy the medulla and repeat (a). Does the heart stop now? Why? Repeat (d) and explain.

Exp. 50—Vaso-constrictors.

Apparatus:—Platinum electrodes, web-board, curare, microscope, induction coil, key, thread, frog.

- (a) Pith brain of a frog and curarize very lightly. This paralyzes the nerve endings in the skeletal but not in the vaso-motor muscles. Place frog, back upward, on frog board, slit open the skin on the thigh a little inside of the median line. Carefully open the muscles without the use of a knife and expose the sciatic nerve. Take great care not to injure the blood vessels and do not stretch the nerve. Place a loose ligature under the nerve. Repeat for the other leg. Spread web of the left leg over hole of board—do not stretch it too tightly and keep it moist on both sides.
- (b) With low power observe the rate of circulation in a small vessel. Place the sciatic nerve of this leg on the electrodes and with repeated induction shocks just perceptible to the tongue stimulate for 2 or 3 seconds. Results? Explain. What sort of nerve fibers were here stimulated? Do not exhaust the nerve; let there be long periods of rest between the successive stimulations.
- (c) Now put the right sciatic nerve on the electrodes and while observing the same web as in (b) stimulate gently. Result? What kind of fibers were now stimulated? What sort of action is this? Prove it by cutting the left sciatic and stimulating the right nerve. Result? Explain.
- (d) Release the web for a few minutes. Place it in position, observe rate. With a brush apply a little adrenalin solution and note any difference. Reason?

Exp. 51—Vaso-motor Mechanism.

Apparatus:—Frog board, web board, a stand, microscope, two small dishes, two frogs.

This experiment must be worked as quickly as possible. Avoid loss of blood—cut no more than is necessary; if the animal bleeds much, the results will be wrong. Two frogs of same size; destroy the brain of one and the brain and cord of the other. Be sure that the cord is destroyed; if it is, the frog will not jerk its leg when the toes are pinched (try this 5 minutes after frog has been pithed). Do not operate until you are sure of this. Expose the heart and intestines (cut a little to one side of the median line) in both frogs. Compare them as follows:

1. Changes in color of the heart during systole and diastole.
2. Fullness and force of the heart action.
3. The condition of the blood vessels on the stomach and intestine.
4. Place the frog on web board and observe circulation in the web (low power of microscope).
5. Suspend the frogs, amputate one of the lower limbs (in thigh) and let the blood drop from the stump into a dish. Count the number of drops shed by each frog. Do not let any fluid from the abdomen mix with this blood. Explain in detail all the differences observed.
6. Brush a little adrenalin chloride over the exposed viscera of the cord pithed frog. Note changes in the vessels to which the adrenalin has been applied. Is the heart affected? Also note any increased bleeding when the other hind leg is amputated.

Exp. 52—Vaso-constrictor Fibers in the Cervical Sympathetic.

Apparatus:—Induction coil, key, wire, electrodes, animal holder, rabbit, ether.

Lightly etherize a rabbit; place it on the holder. Cut the hair from the region over the trachea. Make a longitudinal incision through the skin and fascia over the trachea. By blunt dissection separate the mesial borders of the sternohyoid muscles, thereby exposing the trachea. Along side of the trachea and a little dorsal to it, you will find the carotid artery. Feel for the pulsation. Along the carotid artery lie three nerves. The largest and most distant from the artery is the vagus; the next largest lying next to the carotid artery and gray in color is the cervical sympathetic. The third nerve is the smallest, white in color, and lies in between the other two; this is the depressor nerve.

Very carefully pass a loose ligature under the left sympathetic nerve. Hold the left ear up to the light, handling it very gently in order not to cause vaso-constriction or dilation. Notice the size of the blood vessels. With a medium strength interrupted faradic current stimulate the sympathetic and note the result on the blood vessels. Also notice the condition of the pupil of the left eye. When these effects have been carefully observed and the blood vessels of the ear have come back to normal, place two ligatures on the sympathetic nerve and cut the nerve between the ligatures. Note effect on the vessels, also the color of the ear and its temperature as compared with the other ear. Stimulate the central end. Any effect on the vessels? Next stimulate the peripheral end. Effect?

Discuss as fully as possible the functions of the cervical sympathetic as shown by this experiment.

Exp. 53—Action of the Mammalian Heart.

Apparatus:—Animal board, trachea cannula, ether bottle, ether, key, electrodes, induction coil, thread, curved needle, bone forceps and saw, artery clips, artery cannulas, a dog.

- (a) Administer chloretone by stomach tube. While this is taking effect, get all the instruments and apparatus ready. When the animal is nearly under place it on the holder and tie properly. Give ether if necessary. Place the iron pin of the head piece behind the canine teeth and tie the jaws firmly down to the pin. If necessary, clip the hair from over the trachea, and sternum; make a longitudinal cut through skin and fascia over the trachea. Separate the mesial borders of the sternohyoid muscles and expose the trachea. To the side of the trachea will be found the carotid artery and the vagus. Isolate the vessel and nerve on both sides and place a loose thread under each. With scissors make a transverse V-shaped cut in trachea (below larynx) and insert a trachea cannula. Tie a cannula securely by proper ligature but do not attach the compressed air tube until you are ready to open the thorax.
- (b) With a knife make a median incision through the skin and tissue of the thorax down to the end of the sternum. With bone saw and forceps cut through the sternum, being careful to keep on the middle line of the sternum (to avoid cutting the vessels) and keeping the lower blade of the forceps from stabbing into the organs beneath. As soon as the thorax has been punctured, artificial respiration must be begun, but do not inflate the lungs while the operator is in the act of cutting. If bleeding occurs use artery clips. Pull the thorax open gently and tie the retracted walls to the board, thereby exposing the heart. Observe:—
1. Condition of lungs? Any evidence of anthracosis?
 2. Observe pericardium. Take it between your fingers, pull upon it and determine its thickness, strength and inextensibility. What are the functions of this structure? Slit it open and note the moisture on the heart. Source and object?
 3. The two auricles and two ventricles. The aorta and large veins. The coronary vessels.
 4. Palpate the right and left ventricles and note difference. Why? Note that the ventricles harden during systole and soften during diastole.
 5. Note the color of the right and left auricles. Why this difference? Thickness of their walls?

6. During systole the ventricles twist from left to right. Notice position of apex and the auriculo-ventricular groove. Also note the changes in the three diameters of the heart. What does this give rise to?
7. Place your finger on exposed carotid artery and note that the pulse is almost synchronous with the systole. Can you determine in an exposed artery the actual dilation of the artery during the pulse? Can you detect a pulse in the superior or inferior cava?
8. Press down with your finger on the appendix of the left auricle. Can you detect the beating of the mitral valve?
9. Count the rate of beat per minute. Lift up one of the vagi and place on it two ligatures close together. Cut in between the ligatures. Count rate of heart. Stimulate the peripheral end with very weak repeated induction shocks. If no effect, gradually increase the strength of the current, and note the effect on auricles and ventricles. In which phase does the heart stop? Notice the greatly distended condition of the heart and of the large veins. What do you suppose is the condition of the venous pressure during cardiac inhibition? Why? While the heart is in inhibition prove that it is irritable. Which part of the heart, as it escapes, is the first to beat? Stimulate the central end of the cut vagus. Result on heart rate? How brought about?
10. Raise one of the hind legs and expose the sciatic nerve by cutting through the skin in the middle of the posterior surface of the thigh. Cut through the subcutaneous tissue and separate the muscles until you see the sciatic nerve. Apply a ligature as low down as possible, cut below the ligature. Don't stretch the nerve. Count rate of heart; now stimulate the central end with weak faradic current and during stimulation count heart rate. If no effect, try a little stronger current.

11. Make a muscle-nerve preparation of a frog leg and place the cut end of the nerve on the heart. Notice that the muscle contracts at each heart beat (action current).
12. Stop artificial respiration and note color of left auricle. Cause? Note the rate of the heart beat. Resume respiration and notice how soon normal color is restored. Observe rate of heart.
13. Raise the board so that the feet are up. Notice the effect on the rate and on the fullness of heart. Raise the head and observe.
14. Compress the aorta with the fingers and note effect on the dilation of the auricles and ventricles. Compress inferior vena cava.
15. If Exp. 48, *f*, has been performed, you will omit this Exp. Count rate of heart. Apply a few drops of a 1% pilocarpin to the heart. Describe results. When heart is well under the influence of this drug, apply a little $\frac{1}{2}\%$ atropin. After 3 or 4 minutes count rate. Now stimulate the peripheral end of the vagus nerve. Discuss results.
16. Place the electrodes on the heart and for 2 or 3 seconds send a moderate strength faradic current into the heart. Describe the result. What is this condition called? How does it affect circulation? Why? What is its usual outcome? Notice which part of the heart stops last.

Exp. 54—Blood Pressure.

Apparatus:—Same as Exp. 53; manometer with its attachments; 2 kymographs, signal magnet, key, 1% pilocarpin, $\frac{1}{2}\%$ atropin, amyl nitrite, adrenalin; Ringer's solution, buret.

- (a) Administer chloretone. If necessary give a little ether but be careful not to push this too far.
- (b) Set up inductorium for repeated shocks, placing signal magnet in the primary circuit. Smoke two drums.
- (c) The manometer should be half filled with mercury; the float should slide easily along the tube. Connect the central limb of the manometer with the pressure bottle containing a half saturated solution of sodium sulphate. To the T-tube of the manometer attach a piece of pressure tubing, to the other end of which affix the artery cannula. The wash-out tube of the cannula is supplied with a short piece of rubber tubing and a clip. Place the manometer on a stand at such a height that the level of the mercury will be on a level with the carotid artery. Why? Let its pen come in contact with the drum (about $\frac{1}{5}$ above the bottom of the paper). This level is the level of no pressure; adjust the pen of the signal magnet to write on this line and its curve will represent the base line. Do not change the level of this pen.
- (d) Perform tracheotomy (Exp. 53, a); expose the carotid artery and vagus nerve on one side and also the vagus on the other side. Apply loose ligatures to each. Let another student at the same time expose the sciatic nerve on one side and the femoral vein on the other side. To expose the vein proceed as follows: Place your index finger over the inguinal region just at the lower border of the abdomen where the pulse of the femoral artery can be felt. With scissors cut through the skin and fascia. The triangle of Scarpa (Sartorius, Adductor longus, and External oblique M) will be seen, containing, from within out, the femoral vein and artery and anterior crural nerve. Clean the vein of its fascia for the distance of about one inch; apply an artery clip as high up as possible and after this ligature the distal end. Make a V-shaped incision (toward the heart) and insert a cannula, tying it securely.

- (e) When the carotid artery has been freed from its fascia, insert the cannula attached to the pressure tubing. Raise the pressure bottle, open clip on the tube connecting it with the manometer and also the clip just in front of the cannula and the one on the wash-out. Catch the salt solution as it flows out of the wash-out (do not throw the solution away). By this all the air will be driven out of the tubes and cannula. When this has been accomplished, close the wash-out and the cannula clips and cause a pressure of about 90 or 100 mm. in the manometer. Why? Be sure to close the clip on the pressure bottle tube. Why? Now open the clip on the cannula tube and the artery clip. The blood surges against the solution in the cannula and makes its pressure felt on the mercury of the manometer. Read the amount of pressure on the manometer scale. Take two or three inches of tracing. Observe two sets of waves. What are they called? Notice the synchronism of the more frequent curves with the pulse in the carotid artery, and that of the other waves with the respiration. Does the rise occur during inspiration or expiration? Cause?
- (f) Obtain two inches of normal tracing, then stimulate one of the vagi nerves with moderate strength current. If no effect, increase strength of current a little. Obtain a tracing that shows escape from inhibition. Individual tracing; label: "Blood Pressure, dog, Vagus Stimulation." All the other tracings of this experiment are group tracings.
- (g) Place two ligatures on the right vagus nerve, cut between the ligatures. Stimulate the central end of the vagus with weak current. This may cause a rise in blood pressure (pressor effect). Explain. Stimulation with strong current may cause reflex cardiac inhibition. Does this stimulation have any effect on respiration?
- (h) Stimulate the central end of the cut sciatic nerve with repeated induction shocks (moderate strength). The blood pressure generally rises—pressor effect. Next stimulate with single shocks at the rate of one or two per second. It is possible to obtain a depressor effect; this is especially true if the animal is curarized.
- (i) Obtain about two inches of normal tracing on a slow drum and then close the trachea cannula to observe the effect of dyspnea upon blood pressure. At the moment of closing the trachea, press the key of the signal magnet (object?). Observe the condition of the pupil. Effect on blood pressure? Why? On the heart? As soon as the pressure begins to fall, open the trachea and observe effect on pressure. What are some of the causes of this increase in pressure?

- (j) Raise the foot-end of the dog board in such a manner that the carotid artery and the cannula maintain their previous height. By signal magnet indicate on the drum when this is being done. Hold the animal in this position for about $\frac{1}{4}$ minute. Result on blood pressure and on rate of heart beat? Explain. Now lower the foot-end (same precaution) and note result.
- (k) Effect of amyl nitrite. Place a rubber tube on the trachea cannula and the other end of the tube in a wide mouth bottle. In this bottle put a few drops of amyl nitrite and cover the bottle loosely so that the animal will inhale some of the drug. Indicate on the drum by signal magnet. When the blood pressure begins to fall remove the rubber tube from the trachea cannula. The effect of the inhaled amyl nitrite will continue for some time. Why? Explain the fall in blood pressure.
- (l) Effect of adrenalin. Record an inch of normal tracing and then inject into one of the veins (either by hypodermic syringe or from a buret by means of the cannula in the femoral vein) one or two c.c. of adrenalin (1:10,000). Indicate time of injection on the drum. Watch the mercury in the manometer and do not allow it to pass beyond the bend of the manometer tube (Why?). Explain the effect on blood pressure. Was there any bleeding on the cut surfaces of muscles? Why?
- (m) When the blood pressure is fairly high, inject from $\frac{1}{2}$ to 1 c.c. of 1% pilocarpin solution, depending on size of dog. Note on drum by signal magnet. When the effect is quite evident, inject $\frac{1}{4}$ c.c. of 1% atropin. Explain the results.

- (n) Air embolism. Kill the animal either by air embolism or by bleeding (see o); ask the instructor. After obtaining about an inch of normal tracing, force a small amount of air into the femoral vein. Note on the drum. Place a stethoscope on the chest wall and listen for the noise caused by the frothing of the blood in the heart. How long did it take for the blood pressure to fall? Extent of the fall? Explain.
- (o) Insert a cannula in the other carotid artery. Heat about 500 c.c. of physiological salt solution to 40° C. Fill a buret with this and connect with the femoral vein, making sure that all air has been expelled from the cannula. Obtain normal tracing. Open the clip on carotid artery and remove 50 or 75 c.c. of blood (depending on size of dog). Indicate on drum. After a few minutes remove another 50 or 75 c.c., and continue this until the pressure is down to about 50 mm. Then inject warm salt solution in 50 or 75 c.c. lots. Indicate on the drum and be sure not to admit any air into the vein. Describe the results. Bleed the animal to death while a blood pressure is being taken. How much blood was removed before blood pressure began to fall to any extent? Assuming 1/15 of body weight as blood, what % of the total volume of blood is this? How much blood was altogether removed? Why did the injection of salt solution increase blood pressure? Could the life of the animal be saved thereby? Upon what would that depend?

Exp. 55—Circulation Time.

Apparatus:—Buret, stand, clamp, rabbit, ether, methylene blue solution, thread, rubber membrane, artery clip, artery cannula.

- (a) Administer chloretone to a dog or rabbit. Expose the right carotid artery and left external jugular vein. Under the artery place a piece of rubber membrane and between them a piece of white paper.
- (b) Place a clip on the vein and after the vein is well distended with blood apply a ligature an inch or so above the clip. Make a V-shaped incision in the vein between the clip and the ligature. Insert a cannula (pointing towards the heart) securing it with a ligature. Fill the cannula with P.S.S. so that all the air is driven out.
- (c) Fill the buret with methylene blue (saturated sol. in 0.9% NaCl). Having made certain that all the air is out of the rubber tube attached to the buret, connect this with the cannula.
- (d) Open the clip and let about 2 c.c. of the methylene blue flow into the vein. Note the time accurately by watch (use the second hand). Now watch for the appearance of the methylene blue in the artery. Be sure that you have very good light upon the artery. The moment that a darker mass of blood makes its appearance note the time. Which circulation time is this?

Exp. 56—Lymph Hearts.

The lymph hearts of a frog are situated on either side of the posterior end of the urostyle. Pith the brain of a frog. Very carefully remove the skin from over them and study these structures. Is their action dependent on the central nervous system? Compare the rate of contraction of lymph hearts with that of the heart. Any relation?

PART FIVE

Respiration

Exp. 57—Chest Measurements during Respiration.

Apparatus:—A tape line, calipers, co-ordinate paper.

Measure the circumference of the chest at the level of the axillae: (1) with chest wall at rest at the end of quiet expiration; (2) at the end of quiet inspiration; (3) at the end of most forcible inspiration; (4) at end of forcible expiration. Record the results in centimeters in the table below.

With a pair of calipers measure the antero-posterior diameter of the chest at the junction of manubrium and the mesosternum and at the end of the sternum; record the results.

Also measure the lateral diameter.

	Quiet Breathing		Forced Breathing	
	Inspiration	Expiration	Inspiration	Expiration
Circumference				
Diameter at top of Sternum				
Diameter at end of Sternum				
Lateral Diameter				

From the data obtained with the calipers, plot on co-ordinate paper the cross-section of the thorax in the four positions indicated. Let the backbone be the fixed point of all the curves, which are to be drawn the one within the other.

Exp. 58—Respiratory Capacity; the Spirometer.

Apparatus:—A spirometer.

Sterilize the mouth piece with alcohol. Practice breathing through the mouth piece of the spirometer (holding the nose shut) and notice the swing of the hand on the dial; the amount of air exhaled is indicated on the scale.

- (a) Place the spirometer at the zero mark; take a normal quiet breath and, without paying any attention to the spirometer, exhale to the usual extent through the mouth piece. Repeat six times. Take the average. What is this volume called? Note: 1 cu. inch=16.39 c.c.
- (b) Take another breath; expire the tidal air and after noting the scale, exhale as deeply as possible. What is this second quantity called? Take average of three readings.
- (c) Take in as much air as possible by a very forcible inspiration. Expire through the mouth piece, emptying the lungs as completely as possible. Read the scale and repeat twice. What is this quantity called? From the data obtained calculate the complemental air. Record the averages obtained:

Tidal air	c.c.	cu. in.
Supplemental air		
Complemental air		
Vital capacity		

- (d) Count the rate of quiet respiration in a fellow student who does not know that he is being observed. Supposing that the rate of your respiration is the same, from the above data determine the amount of air respired during quiet respiration in one minute, one hour, one day. How much of the expired air is carbon dioxide? Calculate the amount (in liters and in grams) of this gas discharged in 24 hours. How much oxygen (in liters and in grams) is consumed in this length of time? 1 liter oxygen weighs 1.428 g.; 1 liter carbon dioxide weighs 1.968 g.

Exp. 59—Respiratory Movements in Man. The Pneumograph.

Apparatus:—Pneumograph, kymograph, signal magnet, tambour, plug-key, wire.

With the T-tube open adjust the pneumograph around the chest and connect the nozzle with the rubber tube of the tambour. Close the T-tube. By the movements of the thorax the air pressure in the rubber tube of the pneumograph is altered; this is transmitted to the tambour. Record the excursions of the lever on the drum (medium speed) as also time in 3 seconds. Individual tracing; label "Pneumogram." Note the rate of the pulse. The subject should not look at the tracing while it is being taken, his attention being distracted as much as possible by reading.

(a) Quiet respiration. From the tracing obtained determine:—

1. The rate of respiration per minute;
2. The relative duration of inspiration and expiration;
3. The pause between inspiration and expiration; between expiration and inspiration;
4. The relative speed of inspiration at the beginning and at the end of the phase;
5. Also that of expiration;
6. Ratio of heart beat to respiration.

(b) Record 6 or 7 normal respirations and then let the subject thread a needle. Why is his respiration affected? Group tracing.

(c) After obtaining 6 or 7 normal curves, let the subject breathe very deeply and slowly (18 per minute) for $\frac{1}{2}$ minute and notice the curve following this. Cause of this change? Group tracing.

(d) Obtain a small stretch of tracing while the subject is breathing carbon dioxide for a few seconds. Discuss the changes in rate, depth and rhythm. Group tracing.

Exp. 60—Artificial Respiration. Schaefer's Method.

Apparatus:—A bed sheet.

Schaefer's method "consists in laying the subject in the prone position, preferably on the ground, with a thick folded garment underneath the chest and epigastrium; extend the arms forward. The operator puts himself athwart or at the side of the subject, facing his head, and places his hands on each side over the lower part of the back (lowest rib). He then slowly throws the weight of his body forward, to bear upon his own arms, and thus presses upon the thorax of the subject and forces air out of the lungs. This being effected, he gradually relaxes the pressure by bringing his own body up again to a more erect position, but without moving his hands." Repeat this regularly 12 to 15 times a minute. Each student of the group is required to be in turn subject and operator. The subject is to suspend voluntary breathing and to depend altogether upon the artificial respiration. Is this method efficient?

Exp. 61—Factors Influencing the Respiratory Center.

The respiratory center can be stimulated in three ways: chemically, reflexly, and psychically.

- (a) Breathe very deep at the rate of about 18 per minute for about 40 seconds. Immediately after this note the condition of respiration for the next 10 or 20 seconds. What is this called? How can it be explained? What is this condition of the blood called?
- (b) Continue the deep but slow breathing for a longer length of time (2 or 3 minutes). Notice the light-headedness and other disturbances. Also note that a great effort is necessary to keep up forced respiration. Why?
- (c) Place a large paper bag over your mouth and nose and repeat (a) and (b). Note the greater ease with which the forced respiration can be carried on and the absence of apnea. Explain. If this experiment was continued for a long time what would be the result? Why?
- (d) After a quiet respiration note how long you can hold your breath. How long after one deep inspiration? After two minutes of deep but slow respiration? Explain.
- (e) Note how long you can hold your breath. After 4 or 5 minutes' rest sip water through a tube and notice how long respiration is stopped? Explain: What nerve is here concerned? Why is this of very great importance to us? If the experiment does not succeed, proceed as follows: With the tube in your mouth, but not sipping water, hold your breath. When holding it any longer becomes uncomfortable and the "breaking point" is almost reached, begin to sip water. Result? Explain.

Exp. 62—Pulmonary Pressure.

Apparatus:—Water manometer with tubing. Hg manometer, a bottle with a cork and inlet and outlet tubes, nose clip.

- (a) Arrange the apparatus as in Fig. 3; use the water manometer. Place the mouth piece, *a*, (after sterilizing it with alcohol) in the mouth. While breathing quietly through the nose note the excursions of the water in the manometer during inspiration and expiration. Convert the readings into mm. Hg. and record your results in the table below.



Fig. 3.

- (b) Replace the water manometer by a mercury manometer and repeat as in (a) but respire forcibly.
- (c) Close the nose and make a forcible inspiration and expiration. Read scale and record.

Note:—Be careful not to force the mercury out of the manometer.

	Pressure in mm. Hg
Quiet inspiration.....	
Quiet expiration.....	
Forced inspiration.....	
Forced expiration.....	
Forced inspiration with air passages closed..	
Forced expiration with air passages closed..	

Exp. 63—Respiratory Sounds.

Apparatus:—Stethoscope.

- (a) Vesicular Sound. Place stethoscope over the fifth or sixth right intercostal space and listen to the sound made during quiet inspiration, quiet expiration, forced inspiration and forced expiration. Note quality and duration of the sounds.
- (b) Bronchial Sound. With the stethoscope over the trachea or over the right second costal cartilage listen to the sounds produced during inspiration and expiration. Describe.
- (c) Vocal Fremitus. While the subject is counting listen to the sounds produced in the thorax. Are the sounds heard distinctly? Can you feel the chest vibration with your hand?

Exp. 64—The Mechanics of Respiration.

Apparatus:—The Respiration Scheme (Porter).

Notice the following parts of the apparatus:

(1) The wide glass tube with the rubber balloon which represents the thorax and lungs. (2) The tube opening into the lungs represents the trachea; to this is affixed a piece of rubber tubing with a hole which can be closed by the glass rod. (3) The trachea is connected with a manometer containing mercury which registers the pulmonary pressure. (4) One of the glass tubes leads into the pleural cavity and is connected with a water manometer recording the intra-thoracic pressure. This tube can also be opened to the external air by raising the glass rod in the rubber tubing affixed to the glass tubing. (5) The thorax is connected below with a rubber tube to the other end of which is attached a glass cylinder.

In the glass cylinder place some water; elevate the cylinder so as to let the water flow into the thorax to the extent of about $\frac{1}{2}$ or $\frac{3}{4}$ inch. The surface of the water in the thorax represents the diaphragm which can be lowered or raised by lowering or raising the glass cylinder; this corresponds to inspiration and expiration. Do not let the water come in contact with the lung. During the act of expiration the lungs must never be allowed to collapse to such an extent as not to be slightly stretched.

- (a) Open the opening in the tube mentioned under 4 (call this A) raise the glass cylinder till the water almost touches the lungs and then close A. Now with the tracheal aperture (call this B) only partly open, make inspiration and observe:—(1) intra-thoracic pressure, (2) pulmonary pressure, (3) inflation of the lungs. Explain all these changes. Next produce expiration and again observe.
- (b) Observe the same with forced respiration.
- (c) Pneumothorax. Open A. Is there any change? Cause inspiration and expiration and describe results.
- (d) Adjust the apparatus as in (a). Close B almost entirely and note how the intra-thoracic and pulmonary pressures behave during inspiration and expiration. What condition in the animal does this represent? How is respiration affected by it? What effect will this have on the action of the heart and on the flow of blood? In what condition may this be followed by serious results?
- (e) In coughing a deep inspiration is followed by closure of the glottis; by a violent expiration and a sudden opening of the glottis the air is expelled. Demonstrate this on the model. Notice the pressures.
- (f) Sketch the apparatus, naming its various parts.

Exp. 65—The Vagus in Respiration.

Apparatus:—Animal holder, dog, induction coil, key, electrodes, two time markers, tambour, stand, clamps, kymograph, Woulff bottle.

- (a) Administer small dose of chloretone; give ether if necessary. Place the animal on a holder and expose the vagi nerves. Place ligatures under the nerves but do not tie them. To make certain that you have the vagi nerves, stimulate them with repeated induction shocks and watch for cardiac inhibition (disappearance of the pulse in the exposed carotid artery).
- (b) Perform tracheotomy. To one of the limbs of the trachea cannula attach a piece of rubber tubing provided with a screw clamp. Connect the other limb of the cannula with one of the outlets of a Woulff bottle and connect the Woulff bottle by means of a rubber tube with a tambour. Let the lever of the tambour write on a medium speed kymograph. By chronograph record time in two seconds. Place a second signal magnet in the primary circuit of the induction coil in order to indicate the various steps in the experiment.
- (c) To obtain proper excursion of the tambour lever and thus a good tracing, it may be necessary to partly close the free end of the trachea cannula. Obtain a short stretch of normal respiration curve.
- (d) When 6 or 7 movements have been recorded place two ligatures on the left vagus nerve and cut in between them. Indicate on drum by signal magnet. Group tracing. Note effect of this on the rate and depth of respiration.
- (e) Take an inch or two of tracing and then stimulate the central end of cut vagus with a weak interrupted current. Gradually increase the strength of current. What effect? Stimulate peripheral end and discuss results. Individual tracing.
- (f) Expose the sciatic nerve and stimulate the central end with faradic current. This and all following tracings are group tracings.
- (g) Stimulate the superior laryngeal nerve (indicate on drum) by introducing into the larynx a probe wrapped with a little absorbent cotton. Explain the result. Advantage of this reaction?
- (h) Close the trachea cannula (indicate on drum) and study the effect of dyspnea.
- (i) Cut the other vagus nerve (indicate on drum). How is the rate affected? The depth? What relation between these two changes? Observe that these changes are not temporary.
- (j) Bleed the animal, as in Exp. 54 (o), until the animal is dead. Note the various changes in respiration.

Exp. 66—The Influence of Acid and Alkali on the Respiratory Center.

Apparatus:— $\frac{1}{2}\%$ acetic acid, $\frac{1}{2}\%$ sodium carbonate, kymograph, signal magnet, plug-key, stands, clamp holders, dog, ether, 2 burets, tambour.

- (a) Etherize a dog lightly. Record the respiratory movements as in Exp. 65. Expose both femoral veins (Exp. 54, *d*) and insert cannulas. Fill cannulas with 0.9% NaCl (remove all air) and connect with burets. Fill the one buret with $\frac{1}{2}\%$ acetic acid and the other with $\frac{1}{2}\%$ sodium carbonate. Let the signal magnet write below tambour pen.
- (b) Obtain a stretch of normal tracing; medium or slow speed of drum. Now inject two or three c. c. of acetic acid (indicate on drum); if no effect, inject more. When this has produced a marked effect, inject a small quantity of the carbonate until it neutralizes the effect of the acid. Group tracing. Discuss the results of this experiment and show its bearing on the normal activity of the respiratory center.

Exp. 67—Intra-Thoracic Pressure. Phrenic Nerve.

Apparatus:—Manometer with tubing and pinchcock, trachea cannula, bone saw and forceps, thread, key, induction coil, shielded electrodes, dog, cannula.

- (a) Administer chloretone, expose the trachea; insert a cannula. Connect a glass cannula with a water manometer; color the water with eosin. Make a very small cut in the skin over the 4th intercostal space on right side and make a very small aperture through the intercostal muscles. Through this opening gently force the glass cannula, with as little motion as possible, so that its end lies in the pleural space. There should be no leakage around the cannula and the tissues. Note pressure registered during inspiration and expiration. What pressure here measured? Where does this pressure exist? Its origin? Its variations?
- (b) By cutting along the linea alba open up the abdomen so that the action of the diaphragm can be observed. Notice the central tendinous part and the course of the fibers in the muscular portion. Notice the descent of the diaphragm during inspiration (if necessary close the trachea cannula for a few seconds). Shape of diaphragm? Does the central part move during quiet respiration? During forced respiration?
- (c) Open up the thorax according to Exp. 53 (b). Find the phrenic nerves; isolate, but do not cut them. By means of shielded electrodes stimulate one of them with weak induction shocks. Notice the diaphragm. Stimulate the other phrenic.
- (d) For a short period suspend the artificial ventilation of the lungs. Dyspnea will result and the animal will make inspiratory effort; notice the diaphragm. Immediately cut both nerves. Effect? Stimulate the central end of one phrenic; next the peripheral end. Discuss the nature and function of these nerves. Kill the animal by causing delirium cordis (Exp. 53, 16).

Exp. 68—The Reducing and Oxidizing Power of Tissues.

Apparatus:—Methylene-blue solution, ammonium sulphide, hydrogen peroxide, liver, 1% potassium cyanide, test tubes.

- (a) In a test tube place about 5 c.c. of water and 10 drops of H_2O_2 . Is there any gas liberated? Now add to it a piece of liver. What is the result? What substance in the liver is held responsible for this? To what class of substance does this belong? Boil a small piece of liver for 5 minutes and repeat the above. Does the result agree with your answer to the last question?
- (b) In the above experiment what gas is liberated? Devise an experiment to prove this.
- (c) To 5 c.c. of methylene-blue solution add one drop of ammonium sulphide; shake it slightly. The methylene-blue has been reduced to a colorless compound. Shake it thoroughly and let it stand till the next laboratory period if necessary.
- (d) To some methylene-blue which has been decolorized as described above, add about 10 drops of H_2O_2 . Any change visible? Now add a small piece of liver and let it stand at 37 degrees C. for some time and note the change. Explain.
- (e) Repeat (d) with a piece of boiled liver. Why are the results different from those obtained in (d)?
- (f) To 10 c.c. of methylene-blue solution add 2 or 3 small pieces of raw liver. To another 10 c.c. add a piece of boiled liver. In a third test tube place 10 c.c. methylene-blue, a piece of liver and 1 c.c. of potassium cyanide solution. In a fourth test tube put some methylene-blue for comparison. Cork and set all four test tubes aside for $\frac{1}{2}$ hour or more. Shake the test tubes occasionally. Explain results.

PART SIX

Alimentary Canal

Exp. 69—Deglutition.

Apparatus:—Stethoscope.

- (a) This experiment must be conducted in a quiet room. Place the stethoscope over the region of the larynx of a fellow student. While he is taking a swallow of warm water, note the sound and the interval between the swallowing and the hearing of the sound. Now place the stethoscope over the end of the sternum or over the sixth rib a trifle to the left of the spinal column and listen for the sound. Note character of the sound and the length of time taken for the water to enter the stomach.
- (b) Repeat this experiment while the subject is swallowing solid food. How does this deglutition time compare with that found in (a)? Why this difference?

Exp. 70—Secretion of Pancreatic Juice.

Apparatus:—Induction coil, key, electrodes, dog, 0.4% HCl, cannula, artery clip.

- (a) Administer chloretone to a dog. Open up the abdominal cavity and locate the duodenum and pylorus. Locate the pancreas. Slit open the duodenum and locate the papilla of Vater. In this is the opening of the common bile duct and the duct of Wirsung from the pancreas. The ductus Santorini opens about an inch or two farther down. Insert a cannula into the duct of the papilla and place an artery clip on the common bile duct. If pancreatic juice flows, collect it in a dish.
- (b) Expose one of the vagi nerves in the neck, double ligature it and cut between the ligatures. Stimulate the peripheral end with single induction shocks, once per second.
- (c) Place about 15 c.c. of 0.4% HCl in the duodenum and note the result on the flow of pancreatic juice. Explain the results.

Exp. 71—Peristalsis.

Apparatus:—Trachea cannula, barium chloride, calcium chloride, physostigmin, atropin, a rabbit.

- (a) Lightly etherize a rabbit. Perform tracheotomy so that artificial respiration can be instituted if necessary. Keep the animal warm. By cutting along the linea alba expose the small intestines. If spontaneous movements occur, note both the peristaltic and the pendular movements.
- (b) Pinch a quiescent loop and note the contraction and the relaxation (where?) of the intestines. What law is here demonstrated? Repeat with ascending colon.
- (c) To a loop of the small intestines apply a drop of 1% barium chloride. Result? Now apply a few drops of calcium chloride.
- (d) To another loop apply a drop of 1/10% physostigmin. Neutralize this effect by applying 1/10% atropin.
- (e) Examine the mesentery. Are the lacteals visible? Color? If so, you may be able to notice the beaded appearance due to the constrictions at the lymphatic valves. Locate the receptaculum chyli opposite the kidneys.

Exp. 72—Fat Absorption.

If part (e) of Exp. 71 was not successful, perform the following experiment. Chloroform a cat that has been fed two hours previously with cream; open the abdominal cavity and examine the mesentery. Look for the lacteals. Color? Why? Examine the thoracic duct; open it. Any flow? Nature of fluid?

Exp. 73—Rapidity of Absorption and Secretion.

Apparatus:—Potassium iodide in capsules, starch paste, nitric acid, test tubes.

- (a) To 5 c.c. of thin starch paste and 2 c.c. strong nitric acid add a drop of potassium iodide solution. Result? Chemical equation?
- (b) In each of ten test tubes place 3 c.c. thin starch paste and 1 c.c. nitric acid. Let one student of the group swallow a capsule containing $\frac{1}{2}$ gram KI. Rinse your mouth thoroughly. At 2-minute intervals expectorate some of the saliva into one of the test tubes and note how long after swallowing the KI it appears in the saliva. To quicken the flow of saliva, let the student chew a piece of paraffin or rubber. Describe the pathway of the KI in the body.

Exp. 74—Auto-digestion.

One hour after feeding a rabbit or dog kill the animal by chloroform. Ligate the two openings of the stomach and remove it. Open the stomach, catching the contents in a large dish. Note the consistency of the contents and its reaction to litmus and to dimethyl-amino-azobenzol. What does this demonstrate? Rinse the stomach with water and place it in an incubator at 40° C. Note the condition of the mucosa after a sufficient length of time. Explain. How can we explain the absence of this process in the body?

PART SEVEN

Sweat and Urine

Exp. 75—Secretion of Urine.

Apparatus:—Induction coil, key, electrodes, indigo-carmin, 1% urea solution, cannulas, dog.

- (a) Administer chloroform to a dog. Expose the external jugular vein and insert a cannula; connect this cannula with a buret for injection, having carefully filled the cannula with physiological salt solution in order to remove all air. Expose one vagus nerve, ligate it and cut it central to the ligature. Open the abdominal cavity, locate the ureters. Insert a straight cannula in each and connect them to a Y-tube so as to collect the urine. If there is a spontaneous flow of urine, count and record the drops per minute.
- (b) Into the vein inject 20 c.c. of a 1% urea solution. Note rate of urine secretion, after a short latent period.
- (c) Stimulate the peripheral end of vagus. Effect on urine secretion? Why?
- (d) Introduce 5 c.c. of a saturated solution of indigo-carmin into a vein and note how long a time elapses before the pigment is found in the urine.

Exp. 76—Secretion of Sweat.

Apparatus:—Induction coil, key, electrodes, pilocarpin, atropin, dog.

- (a) Administer chloroform to a dog. Expose the sciatic nerve of the left leg, and the femoral vein on the right side. Observe the pad of the left foot; if there is any evidence of sweat, dry the pad. Now stimulate the peripheral end of the cut sciatic nerve with repeated induction shocks and carefully look for droplets of sweat on the pad of the foot. To what class of nerves do the sudoriferous nerves belong?
- (b) Into the vein inject 2 or 3 c.c. of 1/10% atropin; stimulate the sciatic. Result?
- (c) Now inject 1 c.c. of 1% pilocarpin and again stimulate the sciatic. Observe the pad of the foot for sweat. Result? Explain.

J. B. L.

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